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EXAMINER	
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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/402,488
Filing Date: February 16, 2000
Appellant(s): MOLONEY ET AL.

Micheline Gravelle
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 4/18/07 appealing from the Office action mailed 7/12/06.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The amendment after final rejection filed on 10/11/06 has been entered.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is substantially correct. The changes are as follows: the new matter rejection under 35 U.S.C. 112, first paragraph has been withdrawn for reasons set forth below.

WITHDRAWN REJECTIONS

The following grounds of rejection are not presented for review on appeal because they have been withdrawn by the examiner. The new matter rejection of claims 1, 4-10, 12-16, 18-19, and 50-51 under 35 U.S.C. 112, first paragraph, has been withdrawn upon further consideration of the rejection. In a previous Office action, the examiner raised a new matter rejection asserting that the genus of non-human host cells as recited in claim 1 was not adequately supported by the original application.

According to the holding in *Ex parte Parks*, 30 USPQ2d 1234 (Bd. Pat. App. & Int. 1993), "[i]n rejecting a claim under the first paragraph of 35 U.S.C. 112 for lack of adequate descriptive support, it is incumbent upon the examiner to establish that the originally-filed disclosure would not have reasonably conveyed to one having ordinary skill in the art that an appellant had possession of the now claimed subject matter. *Wang Laboratories, Inc. v. Toshiba Corp.*, 993 F.2d 858, 26 USPQ2d 1767 (Fed.Cir. 1993). Adequate description under the first paragraph of 35 U.S.C. 112 does not require *literal* support for the claimed invention. *In re Herschler,* 591 F.2d 693, 200 USPQ 711 (CCPA 1979); *In re Edwards,* 568 F.2d 1349, 196 USPQ 465 (CCPA 1978; *In re Wertheim,* 541 F.2d 257, 191 USPQ 90 (CCPA 1976). Rather, it is sufficient if the originally-filed disclosure would have conveyed to one having ordinary skill in the art

that an appellant had possession of the concept of what is claimed. *In re Anderson,* 471 F.2d 1237, 176 USPQ 331 (CCPA 1973)."

In this case, the instant specification discloses various examples of non-human host cells, *e.g.,* bacterial, yeast, plant, and mammalian host cells (specification at p. 7, lines 3-4) and animal milk and an edible crop (specification at p. 13, lines 27-29). The working examples in the specification demonstrate the use of an *E. coli* host cell (specification at pp. 14-17). Thus, in view of the disclosure of these representative species of the genus of non-human host cells as encompassed by the claims, it is deemed that appellant has sufficiently "conveyed to one having ordinary skill in the art that [] appellant had possession of the concept" of using a non-human host cell in the method of claim 1 and the new matter rejection has been withdrawn.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

Journal Articles

1) Aigner *et al.,* Expression of the Murine Wild-Type Tyrosinase Gene in Transgenic Rabbits. *Transgenic Research* 5:405-411 (1996).
Entered by Appellants with Amendment filed April 21, 2006.

2) Butler *et al.,* Current Progress in the Production of Recombinant Human Fibrinogen in the Milk of Transgenic Animals. *Thrombosis and Haemostasis* 78(1): 537-542 (1997).
Entered by Appellants with Amendment filed April 2 I, 2006.

3) Cameron, E.R. Recent Advances in Transgenic Technology. *Mol. Biotechnol.* 7:253-265, 1997
Entered herewith.

4) Datta *et al.,* Genetically Engineered Fertile Indicia-Rice Recovered From Protoplasts. *Bio/Technology* 736-40 (1990).
Entered by Appellants with Amendment filed September 12, 2006.

5) Duncker *et al.,* Expression Of A Cystine-Rich Fish Antifreeze In Transgenic *Drosophila melanogaster. Transgenic Research* 5:49-55 (1996).
Entered by Appellants with Amendment filed April 21, 2006.

6) *Dunn et al.,* "Aspartic Proteinases," Advances in Experimental Medicine and Biology, Volume 362, Plenum Press, NY, 1995, pp. 1-9.
Entered by Examiner in Office Action mailed April 29, 2004.

7) Dyck *et al.,* Making Recombinant Proteins In Animals - Different Systems, Different Applications. *Trends in Biotechnology* 21:9:394-399 (2003).
Entered by Examiner in Office Action mailed July 12, 2006.

8) Fine *et al.,* Recombinant Carp *(Cyprinus Carpio)* Growth Hormone: Expression, Purification, And Determination Of Biological Activity. *General Comp. Endocrinol.* 89:51-61 (1993).
Entered by Examiner in Office Action mailed December 4, 2001.

9) Hiatt, A., Antibodies Produced In Plants. *Nature* 344:469-470 (1990).
Entered by Appellants with Amendment filed April 21, 2006.

10) Houdebine, L. Production of pharmaceutical proteins from transgenic animals. *J. Biotechnol.* 34:269-287, 1994.
Entered herewith.

11) Janne *et al.,* Transgenic Animals as Bioproducers of Therapeutic Proteins. *Annals of Medicine* 24:273-280 (1992).
Entered by Appellants with Amendment filed April 21, 2006.

12) Lyons *et al.,* Production Of Protein Pharmaceuticals In Transgenic Plants. *Pharmaceutical News* 3(3): 7-12 (1996).
Entered by Appellants with Amendment filed April 21, 2006.

13) Mason H.S. and Arntzen C.J. Transgenic Plants As Vaccine Production Systems. *Tibtech* 13:388-392 (1995).
Entered by Appellants with Amendment filed April 21, 2006.

14) Mitalipov et al. Rhesus Monkey Embryos Produced by Nuclear Transfer from Embryonic Blastomeres or Somatic Cells. *Biol Reproduction* 66:1367-1373, 2002
Entered herewith.

15) Montoliu, L. Gene Transfer Strategies in Animal Transgenesis. *Cloning and Stem Cells* 4:39-46, 2002
Entered herewith.

16) Potrykus, I., Gene Transfer to Cereals: An Assessment. *Biotechnology* 8(6)535-542 (1990).
Entered by Examiner in Office Action mailed July 12, 2006.

17) Ristevski, S. Making Better Transgenic Models. *Mol. Biotechnol.* 29:153-163, 2005
Entered herewith.

18) Sang, Prospects for transgenesis in the chick. *Mech. Dev.*, 121:1179-1186, 2004.
Entered herewith.

19) Sawers, G and Jarsch, M., Alternative Regulation Principles For The Production Of Recombinant Proteins in *Escherichia coli. Appl Microbiol Biotechno146:1-9* (1996).
Entered by Appellants with Amendment filed April 21, 2006.

20) Sigmund, C.D. Viewpoint: Are Studies in Genetically Altered Mice Out of Control? *Arterioscler. Thromb. Vasc. Biol.* 20:1425-1429, 2000
Entered herewith.

21) Smith, K. Gene transfer in higher animals: theoretical considerations and key concepts. *J. Biotechnol.* 99:1-22, 2002
Entered herewith.

22) Vain, Pet *al.,* Transgene Behavior Across Two Generations in a Large Random Population of Trasgenic Rice Plants Produced By Particle Bombardment. *Theor Appl Genet* 105:878-889 (2002).
Entered by Examiner in Office Action mailed f July 12, 2006.

23) Walsh *et al.,* Investigating the Use of The Chymosin-Sensitive Sequence of k-Casein as a Cleavable Linker Site in Fusion Proteins. J. *Biotech.* 45:235-241 (1996).
Entered by Examiner in Office Action mailed April 29, 2004.

24) Yeh *et al.,* Green Fluorescent Protein As A Vital Marker And Reporter Of Gene Expression In *Drosophila. PNAS* 92:7036-7040 (1995).
Entered by Appellants with Amendment filed April 21, 2006.

25) Yonezawa *et al.*, Sensitive Fluormetric Assay for the Activity of Chymosin, *Int J Pept Protein Res* 47:56-61 (1996).
Entered by Examiner in Office Action mailed March 9, 2005.

Patent Documents

1) U.S. Patent No. 5,472,858
Entered by Appellants with Amendment filed April 21, 2006.

2) U.S. Patent No. 5,639,947
Entered by Appellants with Amendment filed April 21, 2006.

3) U.S. Patent No. 5,650,554
Entered by Appellants with Amendment filed April 21, 2006.

4) U.S. Patent No. 5,827,690
Entered by Appellants with Amendment filed April 21, 2006.

5) U.S. Patent No. 5,959,171
Entered by Appellants with Amendment filed April 21, 2006.

6) U.S. Patent No. 6,265,204
Entered by Examiner in Office Action mailed April 29, 2004.

Other Evidence

Rule 132 Declaration of Dr. Moloney (executed April 7, 2006)
Entered by Appellants with Amendment filed April 21, 2006; entered by Examiner as noted in Office Action mailed July 12, 2006.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 4-10, 12-16, 18-19, and 50-51 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for the preparation of a recombinant polypeptide by transforming an *isolated* host cell with an expression vector encoding a fusion protein comprising a chymosin pro-peptide linked to a recombinant protein, culturing the isolated cell *in vitro* to produce the fusion protein and adding a mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide under *in vitro* conditions to the fusion protein to cleave the pro-peptide from the recombinant protein, optionally wherein the mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide is added by co-transforming the isolated host cell and culturing the isolated host cell to co-express the fusion protein and the zymogenic form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide and activating the autocatalytically maturing aspartic protease by treatment at low pH, does not reasonably provide enablement for practicing the claimed methods in any non-human host organism as encompassed by the claims, wherein cleavage of the fusion protein occurs under *in vivo* conditions in the host organism. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

"The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue." *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976). Factors to be considered in determining

whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See MPEP § 2164.01(a). The Factors most relevant to the instant rejection are addressed in detail below. In view of the analysis of the Factors of *In re Wands* as set forth below, it is the examiner's position that undue experimentation would be required for a skilled artisan to make and/or use the entire scope of the claimed invention.

The breadth of the claims: According to MPEP 2164.04, "[b]efore any analysis of enablement can occur, it is necessary for the examiner to construe the claims...and explicitly set forth the scope of the claim when writing an Office action." MPEP 2164.08 states, "[a]ll questions of enablement are evaluated against the claimed subject matter. The focus of the examination inquiry is whether everything within the scope of the claim is enabled. Accordingly, the first analytical step requires that the examiner determine exactly what subject matter is encompassed by the claims" (citation omitted) and "[w]hen analyzing the enabled scope of a claim, the teachings of the specification must not be ignored because claims are to be given their broadest reasonable interpretation that is consistent with the specification."

Regarding claim 1, the instant specification states, "...the fusion protein is produced *in vivo* in the host animal...The chimeric nucleic acid sequences of the invention may be directly introduced into cells or tissues *in vivo* using delivery vehicles such as retroviral vectors, adenoviral vectors and DNA virus vectors. The chimeric nucleic acid sequences may also be introduced into cells *in vitro* using physical techniques such as microinjection and electroporation or chemical methods such as co-precipitation and incorporation of nucleic acid into liposomes. Expression vectors may also be delivered in the form of an aerosol or by lavage" (p. 13, line 37 to p. 14, line 5). In view of this disclosure of the specification, the scope of claim 1, parts a) and b) has been interpreted as encompassing gene transfer in a non-human host animal and producing the fusion protein in the non-human host animal.

The specification further states, "[a]utocatalytic cleavage requires an alteration of the environment of the fusion peptide. This may include alterations in pH, temperature, salt concentrations, the concentrations of other chemical agents or any other alteration resulting in environmental conditions that will permit autocatalytic cleavage of the fusion protein. The environment may be altered by the delivery of the fusion protein into an appropriate cleavage environment. The cleavage environment may be a physiological environment, such as for example in the mammalian stomach, gut, kidneys, milk or blood, or the environmental conditions may be man-made" (p. 12, lines 11-18). In view of this disclosure of the specification, the scope of claim 1, parts c) and d) has been interpreted as encompassing obtaining the fusion protein from the non-human host animal, followed by cleavage of the fusion protein under *in vivo* conditions. That claim 1

part d) is intended as encompassing cleavage under *in vivo* conditions is further evidenced by the limitations of claims 10, 12, 16, and 18.

Also, in view of the disclosure beginning at p. 7, line 37 of the specification, claims 1 and 51 have been interpreted as encompassing gene transfer in a plant, producing the fusion protein in the plant, and cleaving the fusion protein under *in vivo* conditions in the plant.

The enablement provided by the specification is not commensurate in scope with the disclosure of the specification. In this case, the specification is enabling only for a method for the preparation of a recombinant polypeptide by transforming an isolated host cell with an expression vector encoding a fusion protein comprising a chymosin pro-peptide linked to a recombinant protein, culturing the isolated cell *in vitro* to produce the fusion protein and adding a mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide under *in vitro* conditions to the fusion protein to cleave the pro-peptide from the recombinant protein, optionally wherein the mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide is added by co-transforming the isolated host cell and culturing the host cell *in vitro* to co-express the fusion protein and the mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide.

The state of the prior art; The level of one of ordinary skill; The level of predictability in the art: According to MPEP 2164.03, "...what is known in the art provides evidence as to the question of predictability." There is no dispute that

recombinant protein production was successful in the mammary glands of mice, sheep, pigs, cows, rabbits and goats and in some plants, however, the preponderance of the evidence indicates that gene transfer and recombinant protein production in a non-human animal was an unpredictable art at the time of the invention. For example, regarding gene transfer in animals, Dyck et al. (*Trends Biotechnol* 21:394-399, 2003; "Dyck"), which was published well after the time of the invention teaches "the generation of transgenic domestic animals is difficult and often considered a barrier to their application" for recombinant protein expression (p. 396, left column, bottom) and "[t]he technique that has been the most successful in generating transgenic animals is the microinjection of DNA into the pronuclei of fertilised oocytes...The nature of the avian reproductive systems makes this form of gene transfer impossible in poultry. Furthermore, the unpredictability of the site and rate of transgene integration in the host genome and the resulting variation in the transgene expression because of position effects have also proved problematic" (p. 396, paragraph bridging columns 1-2). Sang (*Mech. Dev.*, 121:1179-1186, 2004), which was published well after the time of the invention, acknowledges that transgenesis techniques in poultry species, such as chick, were highly underdeveloped and unpredictable (see, *e.g.*, p. 1179). Mitalipov et al. (*Biol Reproduction* 66:1367-1373, 2002; "Mitalipov") acknowledges that "somatic cell cloning has not yet been accomplished in primates" (p. 1367, column 2, bottom). Even among those animals that have been used to successfully produce recombinant proteins in their milk, Houdebine (*J. Biotechnol.* 34:269-287, 1994) acknowledges that "[g]ene

transfer remains a difficult...task for farm animals. The vectors carrying the genes coding for the proteins of interest are of unpredictable efficiency" (p. 269, abstract).

According to the prior art, the unpredictability of *in vivo* recombinant protein production is in part due to uncontrolled site of transgene integration, which is critical to transgene expression, yet highly unpredictable due to the possibility of integration into a silent locus. The site of integration may also result in altered tissue specificity, although the promoter used behaves differently at its normal chromosomal localization, with neighboring regulatory elements potentially influencing the transcriptional activity of the transgene (Ristevski, *Mol. Biotechnol.* 29:153-163, 2005, see particularly p. 159, columns 1-2). This phenomenon is known as chromosomal position effects, where host genomic sequences surrounding the site of transgene integration can alter the expected expression pattern, resulting in ectopic or undetectable gene expression (Montoliu, *Cloning and Stem Cells* 4:39-46, 2002, see particularly column 1). Another unpredictable factor in the generation of transgenic animals is the control of copy number, which is evidenced by the reference of Ristevski (*supra*), which teaches, "it is difficult to control transgene copy number (usually integration is a singular event with multiple copies integrated in tandem) (p. 159, column 1, bottom). A high tandem copy number results in a gene silencing effect, and further, was undesirable if the effect of a gene dosage is being addressed, as multiple copies will not recapitulate relevant levels of expression. See also Smith (*J. Biotechnol.* 99:1-22, 2002), which acknowledges the entire process of pronuclear injection is random; there is no means through which to predict or control transgene integration such as copy number, copy orientation,

endogenous sequence rearrangement nor site of integration (p. 11, column 2, bottom). The reference of Smith further states the process of producing a transgenic mammal can result in transcriptional failure and the transgene can disrupt and inactivate an endogenous gene (Smith, p. 12, column 1, top). The position effect and site of integration issues with transgenesis are independent of the method used to insert the DNA sequence into the egg, cell or embryo.

With particular reference to cow, pig and sheep, in part, the existence of certain biological factors inhibits the successful production of a transgenic cow, pig and sheep. One major limitation for sheep, cow and pig is the difficulty in visualizing their egg pronuclei, a critical element for pronuclear injection of the transgene. Cow and pig eggs are opaque rendering the pronuclei difficult to visualize, and sheep, while their eggs are not opaque, the pronuclei are not extremely difficult to visualize (see Smith, p. 11, column 2, top). It follows that if the pronuclei can't be viewed, microinjecting sufficient transgene to generate a transgenic animal for recombinant fusion protein production would be highly unpredictable. According to Smith, the nuclei of farm animal eggs, such as cow, sheep and pig, are much smaller than the nuclei of mouse eggs, thus increasing the likelihood of cell death (p. 11, column 2, middle). Other difficulties exist including poorly anchored pronuclei and lack of visible indicators of post-injection egg damage (Smith, p. 11, column 2, middle). The unpredictability had been established in the art *even after the time of the invention*. Well-regulated transgenic expression was not frequently achieved due to poor levels or the complete absence of expression or leaky expression in non-target tissues (see Cameron, *Mol. Biotechnol.* 7:253-265, 1997,

especially p. 256, paragraph bridging columns 1-2). According to Cameron, "[a] feature common to many transgenic experiments is the unpredictable nature of the transgene expression with different transgenic lines produced with the same construct frequently displaying different levels of expression," wherein the factors influencing low expression, or the lack thereof, were not affected by copy number (p. 256, column 2, top). These factors, thus, are copy number independent and integration site dependent, emphasizing the role the integration site plays on expression of the transgene (Cameron, p. 256, column 2, top). Further, Sigmund (*Arterioscler. Thromb. Vasc. Biol.* 20:1425-1429, 2000) stated that the random nature of transgene insertion, resulting in founder mice could contain the transgene at a different chromosomal site, and that the position of the transgene affects expression, and thus the observed phenotype (Sigmund, p. 1426, top).

It is noted that the references of Sang, Matilipov et al., Ristevski, Houdebine, Montoliu, Smith, Cameron, and Sigmund are newly cited herein. However, these references are provided not as a basis for a new grounds of rejection, but rather to support the teachings of Dyck regarding the unpredictability of generating transgenic animals and their use in recombinant protein production and to provide explanations of art-recognized terms used in the reference of Dyck et al. and Vain et al.

Similar issues regarding the unpredictability of recombinant proteins in plants are reported by Vain et al. (*Theor Appl Genet* 105:878-889, 2002; "Vain"), which teaches "transgene expression in plants remains largely unpredictable" (p. 878, column 2, top). According to Vain, "[f]our main factors impaired transgene expression in primary

transgenic plants (To) and their progeny (T,): (1) absence of transgene expression in To plants (41% of lines), (2) sterility of To plants (28% of lines), (3) non-transmission of intact transgenes to some or all progenies (at least 14% of lines), and (4) silencing of transgene expression in progeny plants (10% of lines)" (p. 878, column 1, middle). Vain teaches, "[d]ifferent integration sites, copy numbers and transgenic locus configurations, as well as epigenetic silencing mechanisms, can all contribute to this variability...Experimental procedures such as transformation systems (Agrobacterium vs direct transfer of DNA), construct configuration..., promoters..., coding sequences, terminators, selection strategy..., flanking Matrix Attachment Regions (MARs)...or the plant tissue analysed...have also been reported to influence transgene structure or expression in plants. The multiplicity of these factors, and their interactions, contribute strongly to the unpredictability, variability and instability of transgene expression in plants" (p. 878, column 2, bottom).

Also, Potrykus (*Biotechnology* 8:535-542) teaches that gene transfer in cereals is largely unsuccessful and even the so-called "successful method" has problems (p. 535, right column, top). According to Potrykus, "my personal experience in working towards the genetic engineering of cereals for the last 18 years convinces me that we still have serious problems in front of us. Success requires more than occasional gene transfer into experimentally well suited varieties of some species. It requires routine and efficient gene transfer into any desired variety of any given species, changing the genome only by the addition of one defined gene. It seems to, me that we are really not yet close to such a situation" (p. 535, columns 1-2).

The amount of direction provided by the inventor; The existence of working examples: According to MPEP 2164.03, "[t]he 'amount of guidance or direction' refers to that information in the application, as originally filed, that teaches exactly how to make or use the invention...if little is known in the prior art about the nature of the invention and the art is unpredictable, the specification would need more detail as to how to make and use the invention in order to be enabling." As evidenced by the teachings of the art as noted above, which evidenced a high level of unpredictability in the art, even *after* the time of the invention, the specification should provide "more detail as to how to make and use the invention."

With regard to transgenesis and recombinant protein production in a non-human animal, the specification fails to provide any guidance regarding how to make such an animal. It is acknowledged that the specification provides some guidance with respect to recombinant protein production in a transgenic plant (specification beginning at p. 7, bottom). However, such guidance is purely hypothetical. There is no working example to demonstrate that a transgenic plant can be successfully generated, including one that will achieve *in vivo* cleavage of the fusion protein to release the recombinant protein as required in claim 51, particularly as the specification fails to provide any evidence that a fusion protein as encompassed by the claims can be cleaved by an auto-catalytically maturing aspartic protease in a plant – either by an endogenous protein, or one that is recombinantly produced. While it is noted there is no requirement that the specification disclose a working example of the claimed invention, MPEP 2164.02 makes clear that "[l]ack of a working example, however, is a factor to be considered, especially in a case

involving an unpredictable and undeveloped art." As evidenced by the cited references, gene transfer and recombinant protein production in a non-human animal and plant was clearly an unpredictable and underdeveloped art at the time of the invention. As such, the lack of a working example of practicing the claimed methods using a non-human animal and/or plant has been considered in determining the enablement of the specification in accordance with 35 U.S.C. 112, first paragraph.

The quantity of experimentation needed to make or use the invention based on the content of the disclosure: At the time of the invention, methods for making transgenic animals and plants were known in the prior art. However, in view of the analysis of the relevant Factors of *In re Wands* as set forth above, including the noted references above that support the position that these methods were underdeveloped and highly unpredictable, the experimentation required to make the full scope of the claimed invention was *not* merely a considerable amount of routine experimentation, but required undue experimentation.

Thus, appellant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). It should be noted that, although the examiner has

indicated enabled subject matter, this is no indication that such is supported by the instant specification, claims, and drawings as originally filed.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claim(s) 1, 4, 6-9, 13, 15, 19, and 51 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Ward et al. (US Patent 6,265,204 B1; "Ward") in view of Walsh et al. (*J Biotech* 45:235-241; "Walsh") and Yonezawa et al. (*Int J Pept Protein Res* 47:56-61 ; "Yonezawa"). The claims are drawn to a method for the preparation of a recombinant fusion protein by recombinantly expressing a full-length chymosin pro-peptide fused to a heterologous polypeptide and cleaving the fusion protein with an aspartic protease.

The reference of Ward teaches a nucleic acid encoding a fusion protein, wherein the nucleic acid encodes (from the 5'-end) a signal sequence, a secreted polypeptide, a cleavable linker, wherein the cleavable linker is a chymosin pro-sequence, and two or more desired polypeptides (see particularly claim 17 and columns 7-8, especially column 7, lines 47-48 and column 4, lines 24-32). Ward teaches that upon construction of the fusion nucleic acid, it is inserted into an expression vector comprising regulatory sequences that are functional in the host to be transformed, including transcriptional

regulatory sequences and transcriptional start and stop sequences (column 11, lines 40-59). Ward teaches the fusion protein is produced by transforming an appropriate host cell with the fusion expression vector and culturing the transformant (column 13, beginning at line 16) followed by cleavage of the fusion protein using, *e.g.,* an endoproteinase (column 14, lines 15-34). Ward teaches cleavage of the fusion protein can occur *in vivo,* citing the use of a host cell expressing a KEX2 protease and co-expressing the fusion protein with a corresponding KEX2 cleavable linker, wherein the fusion was cleaved without requiring prior purification of either the fusion or KEX2 polypeptide and a separate cleavage reaction (Example 3, columns 23-26). Ward acknowledges that recombinant experession of chymosin was known in the art at the time of the invention (*e.g.,* column 1, lines 13-19 and lines 36-40).

Ward does not expressly teach the use of an autocatalytically maturing aspartic protease, particularly chymosin, to cleave the chymosin pro-peptide cleavable linker sequence in the recombinantly produced fusion protein.

At the time of the invention, one of ordinary skill in the art would have recognized that chymosin is an appropriate endoproteinase for cleaving a fusion protein with an N-terminal chymosin pro-peptide sequence as evidenced by Walsh. According to Walsh, in the cleavage of a fusion protein, "[a] specific protease must be used to limit proteolysis to the linker site only, and this site must be designed so that the recognition sequence is accessible to the protease" (p. 235, column 2, middle). Walsh teaches Phe-Met at positions 105 and 106, respectively, of the chymosin substrate κ-casein is a specific chymosin cleavage site and chymosin is the most specific of the aspartyl

proteases (p. 236, column 1, top). Walsh teaches expression of a fusion protein having a linker comprising a Phe-Met chymosin cleavage site (p. 236, column 2 to p. 237, Figures 1-2). Walsh teaches "specific" cleavage of the fusion protein at the Phe-Met site at pH 4 and pH 6.8 by addition of mature chymosin (pp. 236, column 1, top and p. 240, column 1 and Table 1).

The reference of Yonezawa teaches mature chymosin cleaves a peptide (substrate II) specifically at a Phe-Met site at the P1-P1' positions (see particularly p. 58, Table 1). While it is noted that the results of Yonezawa are obtained with short peptides, it is noted that Walsh teaches that their results are "[i]n agreement with observations of chymosin activity on model peptide substrates" (p. 241, left column, top).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to combine the teachings of Ward, Walsh, and Yonezawa for practicing the method for fusion protein preparation and cleavage of Ward using mature chymosin as the endoproteinase cleaving agent, optionally co-expressing chymosin with the fusion protein to effect *in vivo* cleavage in the expression host. One would have been motivated to use chymosin as the fusion protein-cleaving agent in the method of Ward because Walsh teaches that a Phe-Met site is the specific cleavage site of κ-casein, chymosin is the most specific of all aspartyl proteases, and both Walsh and Yonezawa demonstrate that chymosin can cleave a Phe-Met site. One would have been motivated to optionally co-express chymosin with the fusion protein to effect *in vivo* cleavage in the expression host because this would have eliminated a separate step of contacting the fusion protein with a chymosin polypeptide and in essence would

have "automated" a manual process, each of which is a proper rationale for obviousness according to MPEP 2144.04.II and 2144.04.III. One would have a reasonable expectation of success for practicing the method for fusion protein preparation and cleavage of Ward using mature chymosin as the fusion protein-cleaving agent, optionally co-expressing chymosin with the fusion protein to effect *in vivo* cleavage in the expression host, because of the results of Ward, Walsh, and Yonezawa. Therefore, claims 1, 4, 6-9, 13, 15, 19, and 51, drawn to the methods as described above would have been obvious to one of ordinary skill in the art.

Claim(s) 5 is rejected under 35 U.S.C. 103 (a) as being unpatentable over Ward in view of Walsh and Yonezawa as applied to claims 1, 4, 6-9, 13, 15, 19, and 51 above, and further in view of Fine et al. (*Gen Comp Endocrinol* 89:51-61; "Fine"). Claim 5 limits the recombinant protein to hirudin or carp growth hormone.

Ward, Walsh, and Yonezawa disclose the teachings as described above. Additionally, Ward teaches the disclosed method can be used to express a fusion protein comprising an affinity epitope or tag for purification of the fusion protein (see column 10, top). None of Ward, Walsh, or Yonezawa teaches or suggests a method for producing a recombinant carp growth hormone.

Fine teaches the cDNA sequence of cGH has been isolated and characterized (page 52, left column, bottom to right column, top). Fine teaches the recombinant expression of carp growth hormone (cGH) using Escherichia coli as an expression host (p. 52, right column) and purification of the resulting cGH, which required no less than

two chromatographic steps to obtain a purified cGH preparation (p. 55, column 2 to p. 56, column 1).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Ward, Walsh, Yonezawa, and Fine for the method of Ward with cGH as the protein of interest produced as a fusion protein with an affinity tag with a chymosin pro-peptide linker. One would have been motivated to practice the method of Ward with cGH as the desired protein, optionally comprising an affinity epitope or tag, in order that: 1) the cGH protein is expressed by a biologically relevant host cell, *i.e.*, a eukaryotic cell, rather than a prokaryotic cell; and 2) to allow single chromatographic affinity purification of the protein. One would have a reasonable expectation of success for the method of Ward with cGH as the protein of interest produced as a fusion protein with an affinity tag with a chymosin pro-peptide linker because of the results of Ward, Walsh, Yonezawa, and Fine. Therefore, claim 5, drawn to the method as described above would have been obvious to one of ordinary skill in the art at the time of the invention.

Claim(s) 14 and 50 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Ward, Walsh, and Yonezawa as applied to claims 1, 4, 6-9, 13, 15, 19, and 51 above and further in view of Dunn ("Aspartic Proteinases", Advances in Experimental Medicine and Biology, Volume 362, Plenum Press, NY, 1995, pp. 1-9; "Dunn"). Claim 14 limits the aspartic protease added in step c) to an aspartic protease that is

heterologous to the chymosin pro-peptide and claim 50 limits the aspartic protease to pepsin.

Ward, Walsh, and Yonezawa disclose the teachings as described above. Yonezawa further teaches substrate II, which has the sequence Leu-Ser-Phe-Met-Trp-Leu, is cleaved by both chymosin and pepsin and is "hydrolyzed by pepsin at nearly the same rates for chymosin" (p. 58, Table 1 and p. 61, left column, top). None of Ward, Walsh, or Yonezawa teaches cleavage of a fusion protein comprising a chymosin pro-peptide using a heterologous aspartic protease including pepsin.

Dunn is cited as showing that a plurality of aspartic proteases, including pepsin, have the ability to proteolytically cleave a recognition site having Phe in the P1 position and p-NO_2-phenylalanine (Nph) in the P1' position, which, according to Dunn "fit the primary specificity of most members of the aspartic proteinase family well" (p. 3, middle and p. 5, Table 1).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Ward, Walsh, Yonezawa, and Dunn for practicing the method for fusion protein preparation and cleavage as taught by Ward using mature pepsin as a cleaving agent. One would have been motivated to use pepsin as the cleaving agent in the method of Ward because of the teachings of Dunn, which teaches that pepsin can cleave a substrate with Phe in the P1 position and the teachings of Yonezawa that pepsin has a nearly equivalent catalytic specificity for a peptide that is also cleaved by chymosin. See particularly MPEP 2144.06, which acknowledges that substituting equivalents known for the same purpose is a basis for motivation for obviousness. One

would have had a reasonable expectation of success for practicing the method for fusion protein preparation and cleavage of Ward using mature pepsin as a cleaving agent because of the results of Ward, Walsh, Yonezawa, and Dunn, particularly the teachings of Dunn, which show that pepsin can cleave a peptide having Phe at the P1 position, the teachings of Yonezawa, which show that chymosin and pepsin cleave the same substrate peptide, and Walsh , which teaches that results obtained for mature chymosin cleavage of a full-length fusion protein are "[i]n agreement with observations of chymosin activity on model peptide substrates" (p. 241, left column, top). Therefore, claims 14 and 50, drawn to the method as described above would have been obvious to one of ordinary skill in the art at the time of the invention.

(10) Response to Argument

Scope of Enablement Rejection Under 35 U.S.C. 112, First Paragraph

Beginning at p. 13 of the Brief, appellant argues the breadth of the claims is commensurate in scope with the specification. Specifically, appellant argues the specification "provides ample disclosure to guide the skilled artisan in the recombinant production of a protein in non-human host cells," including: 1) numerous expression vectors for effecting expression of the fusion protein in a host cell; 2) methods for introducing an expression vector into a host cell; 3) exemplary methods for recovering a purifying the recombinant protein of interest; and 4) working examples illustrating the claimed methods.

Appellant's argument is not found persuasive. It should be noted that the issue of enablement with respect to the recombinant expression of the recited fusion protein in a bacterial, yeast, or insect cell is not at issue. The issue at hand is whether the specification enables the full scope of the claimed invention, the breadth of which, as noted above, includes, *inter alia*, transgenesis of any plant or non-human animal and recombinant expression of a desired transgene to effect production of a fusion protein in the plant or non-human animal. The breadth of claim 1 further encompasses obtaining the fusion protein from the non-human host animal, followed by cleavage of the fusion protein under *in vivo* conditions. According to the specification, such *in vivo* conditions include a target organ, tissue, or bodily fluid, such as the stomach, gut, kidneys, milk, or blood (specification at p. 13, lines 7-13). The breadth of claim 51 further encompasses cleavage of the fusion under *in vivo* conditions in a plant. That the claims are intended to encompass such embodiments are supported by the disclosure of the specification as noted above. At least for the reasons set forth above in the detailed analysis of the relevant Factors of *In re Wands,* it is the examiner's position that the specification fails to enable the full scope of the claimed invention.

Beginning at p. 14 of the Brief, appellant argues the state of the art of recombinant protein production was advanced. Appellant notes recombinant protein production *per se* is not the focus of the invention and thus the enablement rejection is founded on an improper evaluation of the nature of the invention. According to appellant, the state of the art of recombinant protein production in a non-human host at the time of the invention was "advanced and predictable." Regarding the state of the art

of recombinant protein production in animal cells, appellant relies on the references of Dyck (*supra*), Aigner et al., Butler et al., US Patent 5,959,171, US Patent 5,827,690, and Jänne et al. Regarding the state of the art of recombinant protein production in plant cells, appellant relies on the references of Hiatt, Mason & Arntzen, Lyons et al., US Patent 5,650,554, and US Patent 5,639,947. According to appellant, these references provide evidence that as of the invention's priority date, the state of the art regarding recombinant protein production in an animal and plant was "quite advanced" and "was well within the capabilities of the skilled artisan."

Appellant's argument is not found persuasive. While appellant argues recombinant protein production is not the focus of the invention, it is noted that the breadth of the claims encompasses recombinant protein production in animals and plants, which is undisputed by appellant and is supported by a broad, but reasonable interpretation of the claims in light of the specification. According to MPEP 2164.08, "...it is necessary for the examiner to make sure the full scope of the claim is enabled." In this case, because the claims encompass *in vivo* recombinant protein production in plants and animals and *in vivo* cleavage of the fusion protein, the specification should enable the full scope of the claimed invention.

There is no dispute that the state of the art regarding protein production in an isolated, cultured host cell was advanced at the time of the invention. As such, enablement with respect to the recombinant expression of the recited fusion protein in a bacterial, yeast, or insect cell is not at issue. However, contrary to appellant's position, the state of the art regarding recombinant protein production *in an animal or plant* was

neither "advanced" nor "well within the capabilities of the skilled artisan" at the time of the invention, which is evidenced by the references of Dyck, Sang, Mozdziak, Malipov et al., Houdebine, Vain, Potrykus, Ristevski, Montoliu, Smith, Cameron, and Sigmund, the relevant teachings of which are set forth above.

There is no dispute that representative examples of recombinant transgenic protein production in rabbits, sheep, goats, cows, pigs, and mice were well-known at the time of the invention. Appellant cites various references describing recombinant protein production in these animals at the time of the invention in support of a position that recombinant protein production in animals required no more than routine experimentation. See Aigner et al., Butler et al., US Patent 5,959,171, and US Patent 5,827,690. However, it appears that these references are "anecdotal" in the sense that they disclose experimental evidence of the production of one or two different transgenic animals expressing a single recombinant polypeptide. In this case, it would appear that the more generalized references cited by appellant, *i.e.,* Dyck and Jänne, are more relevant to establishing the state of the art at the time of the invention.

As noted above, the reference of Dyck teaches, "the generation of transgenic domestic animals is difficult and often considered a barrier to their application" for recombinant protein expression (p. 396, left column, bottom). The reference further teaches, "The technique that has been the most successful in generating transgenic animals is the microinjection of DNA into the pronuclei of fertilised oocytes...The nature of the avian reproductive systems makes this form of gene transfer impossible in poultry. Furthermore, the unpredictability of the site and rate of transgene integration in

the host genome and the resulting variation in the transgene expression because of position effects have also proved problematic" (p. 396, paragraph bridging columns 1-2). Again, it should be noted that the reference of Dyck was published well after the time of the invention.

Also, the reference of Jänne (cited by appellant) discloses, "[e]ven though various groups have produced transgenic farm animals, the progress in this field has not been nearly as dramatic as in the generation of transgenic mice. The reasons are obvious: the pregnancy is long, the litter size is small, the availability of fertilized eggs is limited, and technical difficulties are encountered in the microinjection technique. The latter include difficulties in visualizlng the pronuclei due to deposition of opaque material in the cytoplasm of oocytes in some species (pigs, and cattle)" (p. 274, column 1, bottom), which corroborates the teachings of Ristevski, Montoliu, Smith, Cameron, and Sigmund as set forth above. The reference further teaches, "...the production of transgenic bioreactors is still in its infancy and we eagerly await commercial applications...There are, however, many challenges and unsolved problems both concerning the generation of the transgenic bioproducers...In any event, the production of transgenic farm animals is a major effort both costwise and workwise" (p. 278, column 2, bottom).

As such, at least in view of appellant's cited references of Dyck and Jänne, a skilled artisan would have recognized that the generation of transgenic animals and recombinant protein production using such animals is not advanced, well-developed, or routine in the art.

Also, there is no dispute that representative examples of recombinant transgenic protein production in plants was well-known at the time of the invention. However, as noted above, the reference of Vain teaches "transgene expression in plants remains largely unpredictable" (p. 878, column 2, top) and that "[d]ifferent integration sites, copy numbers and transgenic locus configurations, as well as epigenetic silencing mechanisms, can all contribute to this variability...Experimental procedures such as transformation systems (Agrobacterium vs direct transfer of DNA), construct configuration..., promoters..., coding sequences, terminators, selection strategy..., flanking Matrix Attachment Regions (MARs)...or the plant tissue analysed...have also been reported to influence transgene structure or expression in plants. The multiplicity of these factors, and their interactions, contribute strongly to the unpredictability, variability and instability of transgene expression in plants" (p. 878, column 2, bottom). As such, at least in view of the reference of Vain, a skilled artisan would have recognized that the generation of transgenic plants and recombinant production using such plants is not advanced, well-developed, or routine in the art.

Further, as noted above, claims 10, 12, 16, 18, and 51 require *in vivo* cleavage of the fusion protein to release the recombinant protein and the specification fails to provide any evidence that a fusion protein as encompassed by the claims can be cleaved by an auto-catalytically maturing aspartic protease in a non-human animal or plant – either by an endogenous protein, or one that is recombinantly produced.

Beginning at p. 19, top of the Brief, appellant argues the specification should not disclose what is well-known in the prior art. According to appellant, information regarding protein production in host cells need not be disclosed in the specification.

Appellant's argument is not found persuasive. The examiner acknowledges "[a] patent need not teach, and preferably omits, what is well known in the art." *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). However, as evidenced by the teachings of the art of record, including references that were published well after the time of the invention, the generation of transgenic plants and non-human animals and recombinant protein production using such plants and animals was *not* routine or well-known, and thus the specification should provide "more detail as to how to make and use the invention." See particularly MPEP 2164.03, which states, "[t]he 'amount of guidance or direction' refers to that information in the application, as originally filed, that teaches exactly how to make or use the invention...if little is known in the prior art about the nature of the invention and the art is unpredictable, the specification would need more detail as to how to make and use the invention in order to be enabling." However, the specification fails to provide sufficient guidance and direction, particularly as the nature of the invention and the art is unpredictable.

Beginning at p. 19, middle of the Brief, appellant argues the level of skill in the art was advanced at the time of the invention as evidenced by the teachings of the cited references and no evidence of record would suggest otherwise.

Appellant's argument is not found persuasive. As noted above, at least in view of the teachings of the references of Dyck and Jänne (both cited by appellant) and the

teachings of the references of Ristevski, Montoliu, Smith, Cameron, Sigmund, Vain, and Potrykus, the state of the art regarding the generation of transgenic plants and non-human animals and recombinant protein production using such plants and animals was *not* advanced. As acknowledged by Jänne (cited by appellant), "...the production of transgenic bioreactors is still in its infancy." In this case, in view of the preponderance of the evidence presented by the art of record, the generation of transgenic plants and animals and the use of such plants and animals for recombinant protein production was *not* routine and was highly unpredictable.

Beginning at p. 19, bottom of the Brief, appellant argues "[t]he evidence discussed above...indicates that there is a high level of predictability in the art," citing the results of Vain, which, according to appellant, demonstrates a high level of predictability in recombinant protein production in plants. Appellant argues the level of predictability in the art is supplemented by the amount of guidance provided in the specification, pointing to p. 9 of the specification, which appears to disclose various generic teachings regarding the generation of transgenic plants and recombinant protein production using such plants.

Appellant's argument is not found persuasive. As noted above, in view of the preponderance of the evidence presented by the art of record, the generation of transgenic plants and animals and the use of such plants and animals for recombinant protein production was *not* routine and was highly unpredictable. While appellant attempts to rely on the results of Vain to support their position, the teachings of Vain would appear to support the contrary position. As noted above, Vain discloses,

"transgene expression in plants remains largely unpredictable" (p. 878, column 2, top). According to Vain, "[f]our main factors impaired transgene expression in primary transgenic plants (To) and their progeny (T,): (1) absence of transgene expression in To plants (41% of lines), (2) sterility of To plants (28% of lines), (3) non-transmission of intact transgenes to some or all progenies (at least 14% of lines), and (4) silencing of transgene expression in progeny plants (10% of lines)" (p. 878, column 1, middle). Vain teaches, "[d]ifferent integration sites, copy numbers and transgenic locus configurations, as well as epigenetic silencing mechanisms, can all contribute to this variability...Experimental procedures such as transformation systems (Agrobacterium vs direct transfer of DNA), construct configuration..., promoters..., coding sequences, terminators, selection strategy..., flanking Matrix Attachment Regions (MARs)...or the plant tissue analysed...have also been reported to influence transgene structure or expression in plants. The multiplicity of these factors, and their interactions, contribute strongly to the unpredictability, variability and instability of transgene expression in plants" (p. 878, column 2, bottom).

In this case, as evidenced by the teachings of the art of record, including references that were published well after the time of the invention, the generation of transgenic plants and non-human animals and recombinant protein production using such plants and animals was *not* routine or well-known, and thus the specification should provide "more detail as to how to make and use the invention." See particularly MPEP 2164.03, which states, "[t]he 'amount of guidance or direction' refers to that information in the application, as originally filed, that teaches exactly how to make or

use the invention...if little is known in the prior art about the nature of the invention and the art is unpredictable, the specification would need more detail as to how to make and use the invention in order to be enabling." However, the specification fails to provide sufficient guidance and direction to enable the full scope of the claimed invention, particularly as the nature of the invention and the art is unpredictable. The specification fails to provide any guidance and direction regarding the production of even a single transgenic non-human animal and, while the specification provides some general guidance and direction for generating a transgenic plant, in the absence of even a single working example of such, the specification provides no expectation that such a plant can be generated and used for recombinant protein production in accordance with the claimed methods.

Beginning at p. 20 of the Brief, appellant argues the specification discloses two working examples that further enable the claimed methods. Appellant notes Examples 1 and 2, which disclose recombinant protein production in a bacterial host cell – specifically an *E. coli* host cell. According to appellant, "[b]ecause the specification describes several methods for practicing the claimed invention, the enablement requirement is satisfied."

Appellant's argument is not found persuasive. There is no dispute that the specification discloses a method for production of a fusion protein in an *E. coli* host cell. However, the scope of host cells of the claimed method is not limited to *E. coli* as an expression host. Instead, the claims are intended to encompass generation of a transgenic non-human animal and plant and using such animals and plants for

recombinant protein production. According to MPEP 2164.01(b), "[a]s long as the specification discloses at least one method for making and using the claimed invention *that bears a reasonable correlation to the entire scope of the claim*, then the enablement requirement of 35 U.S.C. 112 is satisfied. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970)" (emphasis added). In this case, a skilled artisan would recognize that transforming an *E. coli* with a recombinant DNA and producing a protein using that transformant, which was routine in the art at the time of the invention, *does not* bear a reasonable correlation to the generation of transgenic non-human animals and plants and the use of such animals and plants in the production of recombinant proteins. Even assuming *arguendo* steps a) and b) of claim 1 were limited to recombinant protein production using an *E. coli* host cell, it is noted that part d) of claim 1 encompasses cleavage of the fusion protein *in vivo* (see, *e.g.,* claims 10, 12, 16, and 18), and neither the specification nor the prior art provide guidance for effecting cleavage of the fusion protein under any *in vivo* conditions, which, according to the specification, encompasses mammalian stomach, gut, kidneys, milk or blood (specification at p. 12, lines 11-18).

Beginning at p. 21, bottom of the Brief, appellant argues there is no evidence that an undue quantity of experimentation would be required to practice the claimed methods. According to appellant, the examiner has not cited any evidence to support a requirement for undue experimentation. Appellant argues the references cited by the examiner, *i.e.,* Dyck, Vain, and Potrykus, fail to suggest an undue experimentation is required and instead actually support appellant's position of the advanced state of the

art, because the references address issues that have arisen as recombinant protein production has moved out of the research laboratory into the commercial world. Addressing the reference of Dyck, appellant argues the teachings of Dyck are "purely commercial considerations that have no bearing on the enablement of the claimed methods"; the reference cites numerous categories of successful transgenic protein production and methods for producing transgenic animals; and that the teachings of Dyck are "hardly evidence of non-enablement."

Appellant's argument is not found persuasive. Contrary to appellant's position, the references cited by the examiner support the position that the art of generating transgenic animals and plants and using such animals and plants for recombinant protein production was not routine in the art and was highly unpredictable. As noted above, the reference of Dyck teaches, "the generation of transgenic domestic animals is difficult and often considered a barrier to their application" for recombinant protein expression (p. 396, left column, bottom). The reference further teaches, "The technique that has been the most successful in generating transgenic animals is the microinjection of DNA into the pronuclei of fertilised oocytes...The nature of the avian reproductive systems makes this form of gene transfer impossible in poultry. Furthermore, the unpredictability of the site and rate of transgene integration in the host genome and the resulting variation in the transgene expression because of position effects have also proved problematic" (p. 396, paragraph bridging columns 1-2). Again, it should be noted that the reference of Dyck was published well after the time of the invention.

Also, the reference of Jänne (cited by appellant) appears to further support the examiner's position by disclosing, "[e]ven though various groups have produced transgenic farm animals, the progress in this field has not been nearly as dramatic as in the generation of transgenic mice. The reasons are obvious: the pregnancy is long, the litter size is small, the availability of fertilized eggs is limited, and technical difficulties are encountered in the microinjection technique. The latter include difficulties in visualizlng the pronuclei due to deposition of opaque material in the cytoplasm of oocytes in some species (pigs, and cattle)" (p. 274, column 1, bottom). See also the teachings of the references of Ristevski, Montoliu, Smith, Cameron, and Sigmund as set forth above.

Addressing the reference of Vain, appellant argues the examiner's cited teachings were taken out of context, and thus the reference fails to support the examiner's position of unpredictability in the art of making and using transgenic plants for recombinant protein production.

Appellant's argument is not found persuasive. The examiner acknowledges that the cited teaching of Vain is not the entire sentence from which it is taken. However, it appears that it is appellant and not the examiner who has taken the teaching of Vain out of context as Vain teaches that "transgene expression in plants remains largely unpredictable, *and* there is considerable variation in expression levels and stability between independently transformed plants" (emphasis added, p. 878, right column, top). As such, these statements appear to be independent, *i.e.*, the unpredictability referred to by Vain is not limited to the variation in expression level and stability of transformed plants. Moreover, Vain goes on to state, "[f]our main factors impaired

transgene expression in primary transgenic plants (To) and their progeny (T,): (1) absence of transgene expression in To plants (41% of lines), (2) sterility of To plants (28% of lines), (3) non-transmission of intact transgenes to some or all progenies (at least 14% of lines), and (4) silencing of transgene expression in progeny plants (10% of lines)" (p. 878, column 1, middle) and "[d]ifferent integration sites, copy numbers and transgenic locus configurations, as well as epigenetic silencing mechanisms, can all contribute to this variability...Experimental procedures such as transformation systems (Agrobacterium vs direct transfer of DNA), construct configuration..., promoters..., coding sequences, terminators, selection strategy..., flanking Matrix Attachment Regions (MARs)...or the plant tissue analysed...have also been reported to influence transgene structure or expression in plants. The multiplicity of these factors, and their interactions, contribute strongly to the unpredictability, variability and instability of transgene expression in plants" (p. 878, column 2, bottom). Thus, contrary to appellant's position, the teachings of Vain support a high level of unpredictability in the art of making transgenic plants and using such plants in the production of recombinant proteins.

Addressing the reference of Potrykus, appellant argues the reference "relates to issues encountered on the road to commercialization" and that the reference is a subjective discussion. According to appellant the reference "does not undermine the enablement of the present invention with respect to recombinant protein production in plants."

Appellant's argument is not found persuasive. According to Potrykus, "my personal experience in working towards the genetic engineering of cereals for the last 18 years convinces me that we still have serious problems in front of us. Success requires more than occasional gene transfer into experimentally well suited varieties of some species. It requires routine and efficient gene transfer into any desired variety of any given species, changing the genome only by the addition of one defined gene. It seems to, me that we are really not yet close to such a situation" (p. 535, columns 1-2). Potrykus acknowledges that even the successful method of gene transfer to cereals "has its problems" (p. 535, column 2, top).

It is acknowledged that Potrykus characterizes his discussion as "subjective." However, that the reference is described as "subjective" should not discredit the teachings set forth therein, particularly as any scientific publication is necessarily at least somewhat subjective, based on the author's interpretation of the data presented. Moreover, Potrykus admits experience in the field of genetic engineering of cereals "for the last 18 years" (p. 535, column 1), and thus the "subjective" discussion would appear to be based on a significant level of experience in the field of genetic engineering of cereals.

While appellant takes the position that the references address "purely commercial concerns," nowhere do the references appear to suggest that they are related to "purely commercial concerns" and appellant has provided no evidence to support such a position. Further, it is noted that the prior art reference of Jänne (cited by appellant) would suggest otherwise. According to Jänne, "...the production of transgenic

bioreactors is still in its infancy and we eagerly await commercial applications" (p. 278, column 2, bottom). See also Potrykus, which indicates that transgenic plants have a "*potential* agronomic utility" (p. 535, abstract), thus suggesting that transgenic plants, while having the potential to have agronomic utility, have yet to achieve such utility.

Appellant argues that in citing the references of Dyck, Vain, and Potrykus, the examiner "loses sight of the fact that § 112 does not require an appellant 'to enable one of ordinary skill in the art to make and use a perfected, commercially viable embodiment.'" Appellant notes all that § 112 requires is that the specification enable the claimed invention without requiring undue experimentation, further noting that even considerable experimentation may be required if it is merely routine or if the specification provides a reasonable amount of guidance. Appellant argues that because they have allegedly demonstrated that recombinant protein production in a wide variety of non-human hosts was well-developed at the time of the invention, the instant rejection is improper.

Appellant's argument is not found persuasive. Contrary to appellant's allegation, the examiner has not required a standard of enablement such that a skilled artisan can make and use a "perfected, commercially viable embodiment." Instead, the examiner maintains that the specification should enable the full scope of the claimed invention. As supported by the disclosure of the specification, the claims encompass generation of transgenic non-human mammals and plants and use thereof in the production of recombinant proteins. For reasons noted above, the examiner's position is that the specification fails to enable these embodiments of the claimed invention, particularly as

making and using transgenic non-human animals and plants was not routine and was highly unpredictable as supported by the references of Dyck, Vain, and Potrykus.

Beginning at p. 24, bottom of the Brief, appellant separately addresses the enablement of claim 51, noting the examiner has admitted enablement of claim 51 with respect to bacteria and yeast host cells. According to appellant, the "record evidence of enablement discussed in section B.1...demonstrates that the invention is enabled with respect to...plants." Appellant notes this record evidence includes the references of Hiatt, Mason & Arntzen, Lyons et al., US Patent 5,650,554, and US Patent 5,639,947 and disclosure of the specification at p. 9 and in Figure 1.

Appellant's argument is not found persuasive. The examiner acknowledges that claim 51 is enabled to the extent that the claim recites "bacterial cells" and "yeast cells" as the expression host. However, the examiner maintains that the specification fails to enable the full scope of the claimed invention with respect to "plant cells" as the expression host, which, according to the specification and undisputed by appellant, is intended as encompassing transgenic plants, use thereof for recombinant protein production, and *in vivo* cleavage of the fusion protein. As noted above, there is no dispute that representative examples of recombinant transgenic protein production in plants was well-known at the time of the invention. However, as noted above, the reference of Vain teaches "transgene expression in plants remains largely unpredictable" (p. 878, column 2, top) and that "[d]ifferent integration sites, copy numbers and transgenic locus configurations, as well as epigenetic silencing mechanisms, can all contribute to this variability...Experimental procedures such as

transformation systems (Agrobacterium vs direct transfer of DNA), construct configuration..., promoters..., coding sequences, terminators, selection strategy..., flanking Matrix Attachment Regions (MARs)...or the plant tissue analysed...have also been reported to influence transgene structure or expression in plants. The multiplicity of these factors, and their interactions, contribute strongly to the unpredictability, variability and instability of transgene expression in plants" (p. 878, column 2, bottom). As such, at least in view of the reference of Vain, a skilled artisan would have recognized that the generation of transgenic plants and recombinant production using such plants is not advanced, well-developed, or routine in the art. Further, as noted above, claim 51 requires *in vivo* cleavage of the fusion protein to release the recombinant protein and the specification fails to provide any evidence that a fusion protein as encompassed by the claims can be cleaved *in vivo* in a plant by an auto-catalytically maturing aspartic protease – either by an endogenous protein, and/or one that is recombinantly produced. Moreover, there is not a single working example disclosed in the specification of a transgenic plant expressing a fusion protein as encompassed by the claims with the ability to express an auto-catalytically maturing aspartic protease that can contact and cleave the fusion protein.

Obviousness Rejection Under 35 U.S.C. 103(a): Ward in view of Walsh and Yonezawa

Beginning at p. 26 of the Brief, appellant summarizes case law relevant to obviousness under 35 U.S.C. 103(a). Beginning at the middle of p. 27 of the Brief, appellant argues the cited references fail to teach or suggest the claimed methods and there is no motivation or reasonable expectation of success for practicing the claimed

methods. Addressing the reference of Ward, appellant argues Ward fails to provide specific teachings of how to cleave a bovine chymosin pro-sequence from the fusion protein and does not teach the use of an autocatalytically maturing aspartic protease for fusion protein cleavage; addressing the reference of Walsh, appellant argues the reference does not teach or suggest the use of a chymosin pro-peptide as a fusion protein cleavable linker; and addressing the Yonezawa reference, appellant argues the reference does not teach or suggest the use of an autocatalytically maturing aspartic protease for cleavage of a chymosin pro-peptide in a fusion protein.

Appellant's argument is not found persuasive. At least for the reasons of record, the examiner maintains the position that the combination of cited prior art references teaches or suggests the claimed invention and provides motivation and a reasonable expectation of success for making the claimed invention. It should be noted that there is no dispute that the *combination* of references teaches all limitations of the claimed invention. The findings of the examiner which are not challenged are usually accepted as fact. See *In re Kunzmann*, 326 F.2d 424, 140 USPQ 235 (CPA 1964).

The examiner acknowledges appellant's noted deficiencies of each of the references of Ward, Walsh, and Yonezawa. However, the rejection is not based on any one of the cited references or a subcombination thereof. Instead, the rejection is based on the combination of all of the cited references. One cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Beginning at p. 29, top of the Brief, appellant argues the rejection is based on the incorrect assumption that chymosin will cleave any fusion protein at a Phe-Met bond. Appellant refers to the Moloney Declaration, which explains that the prior art references of Visser et al., which is cited by Walsh, and Schettenkerk et al., which is cited by Visser et al., disclose that a minimum chain length of five amino acids residues including a Ser-Phe-Met-Ala is required for cleavage of a κ-casein polypeptide. Appellant also points to the Moloney Declaration explaining that in Figures 1 and 2 and the working examples that chymosin cleavage did not occur between a Phe-Met bond in a GST-Pro-Hirudin fusion protein and did occur between a Phe-Val and a Phe-Ser bond. In view of this evidence, appellant asserts the Moloney Declaration, the prior art, and the application data demonstrate that chymosin does not cleave at any and all Phe-Met bonds. According to appellant, because one of ordinary skill in the art would not have expected chymosin to cleave all fusion proteins at a Phe-Met bond, the cited references fail to teach the claimed invention. Appellant argues one of ordinary skill in the art would not have had a reasonable expectation of success that an aspartic protease would cleave a fusion protein to release the recombinant polypeptide and did not know whether the aspartic protease would non-specifically cleave the recombinant polypeptide and/or would cleave off too many or too few amino acids around the pro-peptide junction. According to appellant, "[w]ithout an assurance of accurate cleavage, there was no motivation to have employed an aspartic protease as presently claimed."

Appellant's argument is not found persuasive. Initially, it is noted that appellant is arguing against a limitation that is not present in the claims – the examiner's noting of a

Phe-Met junction in a fusion protein was based on the disclosure of Walsh, which specifically discloses this sequence as a chymosin-cleavage site. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

In this case, appellant's position appears to be that chymosin does not cleave at all Phe-Met bonds and thus one of ordinary skill in the art would have no reasonable expectation of success for practicing the claimed invention. MPEP 2143.02 makes clear that absolute predictability is not required, only *some* degree of predictability. In view of the evidence of record, the examiner does not dispute appellant's assertion that chymosin does not cleave at all Phe-Met bonds. However, the issue at hand is not whether chymosin has the ability to cleave at all Phe-Met bonds, but rather would an ordinarily skilled artisan have an expectation of success that chymosin could cleave a chymosin pro-peptide in a fusion protein as taught by Ward.

At the time of the invention, the prior art regarding preferred cleavage sites for chymosin was well-developed as evidenced by the references of Yonezawa and Walsh and also by references cited by Walsh, namely those of Visser et al. and Schettenkerk et al., which is acknowledged by appellant in the Brief at p. 29, second paragraph: "[s]pecifically, the art teaches that a minimum chain length of five amino acid residues including the sequence Ser-Phe-Met-Ala is essential to bring about a cleavage of the Phe-Met bond in κ-casein. (See paragraph 6 of the Moloney Declaration, citing Visser et al. and Schattenkerk et al.)". Thus, in view of the available known cleavage sites for

chymosin as were well-known in the prior art, one of ordinary skill in the art could have readily engineered a cleavage site for a chymosin pro-peptide fused to a recombinant protein of interest with an expectation of successfully cleaving the pro-peptide from the recombinant protein of interest, particularly in view of the successful cleavage at a Phe-Met junction as shown by Walsh and Yonezawa. Even appellant acknowledges that such experimentation was routine in the art at the time of the invention. In the response filed on 1/27/2003, appellant asserted that it is routine in the art to select a pro-peptide from any aspartic protease, fuse that pro-peptide to any recombinant protein of interest, and use any aspartic protease to cleave at the fusion protein junction (response filed on 1/27/2003 at pp. 3-5). As such, one of ordinary skill in the art at the time of the invention would have had a reasonable expectation of success for fusing a chymosin pro-peptide to a recombinant protein of interest having an appropriate chymosin cleavage site and using chymosin to cleave at the fusion protein junction in accordance with the method of Ward. In view of the teachings of the prior art of Walsh and Yonezawa appellant's cited references of Visser et al. and Schattenkerk et al., and further in view of appellant's admission that selecting a pro-peptide, fusing that propeptide to a recombinant protein of interest, and using an aspartic protease as a cleavage agent was routine at the time of the invention, one of ordinary skill in the art would have had at least a *reasonable* expectation of success for making the claimed invention.

Addressing appellant's allegation that "[w]ithout an assurance of accurate cleavage, there was no motivation to have employed an aspartic protease as presently claimed," it is noted that the claims do not require *specific* or *accurate* cleavage of a

fusion protein. As such, appellant is arguing a limitation that is not present in the claims. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). See also MPEP 2145 regarding arguing limitations that are not claimed. In fact, appellant has previously argued that "the claims do not preclude some non-specific cleavage of the heterologous protein" (response filed on 9/18/2001 at p. 6, bottom). In the same response, appellant states, "[a]pplicant has tested many proteins and has not observed *substantial* non-specific cleavage of any of the proteins" (italics added for emphasis, response filed on 9/18/2001 at p. 7, top), thus suggesting that at least *some* non-specific cleavage has been observed.

Even assuming *arguendo* the claims required *specific* or *accurate* cleavage of a fusion protein, as acknowledged by Walsh, chymosin is the most specific of the aspartyl proteases (p. 236, column 1, top), which would have motivated one of ordinary skill in the art to use chymosin to cleave its corresponding pro-peptide, particularly as Walsh teaches, "[a] specific protease must be used to limit proteolysis to the linker site only, and this site must be designed so that the recognition sequence is accessible to the protease" (p. 235, column 2, middle).

Obviousness Rejection Under 35 U.S.C. 103(a): Ward in view of Walsh and Yonezawa and further in view of Fine

Beginning at p. 30, bottom of the Brief, appellant argues the teachings of Fine "do not remedy the inability of Ward, Walsh, and Yonezawa to render obvious the claimed invention" of claim 1, from which claim 5 depends.

Appellant's argument is not found persuasive. There appears to be no dispute that the combination of references teaches all limitations of the claims and provides motivation to combine the teachings of Fine with the teachings of Ward, Walsh, and Yonezawa. The findings of the examiner which are not challenged are usually accepted as fact. See *In re Kunzmann*, 326 F.2d 424, 140 USPQ 235 (CPA 1964).

What is in dispute is whether the invention would have been obvious in view of the combination of Ward, Walsh, and Yonezawa such that the reference of Fine can be further combined with these references. At least for the reasons noted above, the examiner maintains that the invention of claim 1 would have been obvious in view of the combination of Ward, Walsh, and Yonezawa, and the invention of claim 5, which depends from claim 1, would have been obvious in view of Ward, Walsh, and Yonezawa and the further teachings of Fine.

Obviousness Rejection Under 35 U.S.C. 103(a): Ward in view of Walsh and Yonezawa and further in view of Dunn

Beginning at p. 31, middle of the Brief, appellant argues the claimed invention would not have been obvious in view of the teachings of Ward, Walsh, and Yonezawa. Appellant maintains that "there is simply no hint in the prior art of using a mature aspartic protease to cleave a chymosin pro-peptide sequence from a fusion protein to

release a recombinant protein of interest." Appellant argues that although mature aspartic proteases have been shown to cleave specific peptides at specific sites, this does not teach the use of a mature aspartic protease for cleaving the recited fusion protein. Appellant reiterates the position that one of ordinary skill in the art would not have had a reasonable expectation of success that an aspartic protease would cleave a fusion protein to release the recombinant polypeptide and did not know whether the aspartic protease would non-specifically cleave the recombinant polypeptide and/or would cleave off too many or too few amino acids around the pro-peptide junction. According to appellant, "[w]ithout an assurance of accurate cleavage, there was no motivation to have employed an aspartic protease as presently claimed."

Appellant's argument is not found persuasive. There appears to be no dispute that the combination of references teaches all limitations of the claims and provides motivation to combine the teachings of Dunn with the teachings of Ward, Walsh, and Yonezawa. The findings of the examiner which are not challenged are usually accepted as fact. See *In re Kunzmann,* 326 F.2d 424, 140 USPQ 235 (CPA 1964). What is in dispute is whether the invention would have been obvious in view of the combination of Ward, Walsh, and Yonezawa such that the reference of Dunn can be further combined with these references. At least for the reasons noted above, the examiner maintains that an ordinarily skilled artisan would have a reasonable expectation that an autocatalytically maturing aspartic protease, *e.g.* pepsin, would be capable of cleaving a chymosin pro-peptide in view of the combination of Ward, Walsh, and Yonezawa. As

such, the invention of claims 14 and 50, which depend from claim 1, would have been obvious in view of Ward, Walsh, and Yonezawa and the further teachings of Dunn.

While appellant argues that an ordinarily skilled artisan would have no expectation of non-specific cleavage effects, *i.e.,* cleavage at undesired sites and/or cleaving too many or too few amino acid residues around the junction between the pro-peptide and the recombinant protein, the claims are not limited to any particular level of *specific* or *accurate* cleavage of a fusion protein. As such, appellant is addressing a limitation that is not present in the claims. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). See also MPEP 2145 regarding arguing limitations that are not claimed. In fact, as noted above, appellant's previous statements indicate that non-specific cleavage of the fusion protein is encompassed by the claimed method. "The claims do not preclude some non-specific cleavage of the heterologous protein" (response filed on 9/18/2001 at p. 6, bottom). In the same response, appellant states, "[a]pplicant has tested many proteins and has not observed *substantial* non-specific cleavage of any of the proteins" (italics added for emphasis, response filed on 9/18/2001 at p. 7, top), thus suggesting that at least *some* non-specific cleavage has been observed in appellant's own experiments.

Second, MPEP 2143.02 makes clear that absolute predictability is not required, only *some degree* of predictability. In view of the teachings as described above, one of ordinary skill in the art at the time of the invention would have had at least *some degree* of predictability that the fusion protein as taught by Ward could be cleaved by an

autocatalytically maturing aspartic protease other than chymosin, *e.g.*, pepsin. It would appear that even appellant acknowledges at least *some degree* of predictability that the fusion protein as taught by Ward could be cleaved by an autocatalytically maturing aspartic protease other than chymosin. As noted above, in the response filed on 1/27/2003, appellant asserted that it is routine in the art to select a pro-peptide from any aspartic protease, fuse that pro-peptide to any recombinant protein of interest, and use any aspartic protease to cleave at the fusion protein junction (response filed on 1/27/2003 at pp. 3-5). As such, one of ordinary skill in the art at the time of the invention would have had a reasonable expectation of success for fusing a chymosin pro-peptide to a recombinant protein of interest having an appropriate chymosin cleavage site and using an autocatalytically maturing aspartic protease other than chymosin, *e.g.* pepsin, to cleave at the fusion protein junction in accordance with the method of Ward.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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